Regional blood flows in the goat latissimus dorsi muscle before and after chronic stimulation

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Regional blood flows in the goat latissimus dorsi muscle before and after chronic stimulation. J. Appl. Physiol. 81(6): 2365–2372, 1996.—Latissimus dorsi muscle (LDM) regional blood flows were determined in anesthetized goats by using colored microspheres under noncontracting and contracting conditions, either before or after 8–10 wk of chronic muscle stimulation. Surgical dissection of the LDM, leaving only the thoracodorsal artery to supply the muscle, did not alter regional noncontracting blood flows but significantly reduced the normal hyperemic response to muscle contraction in muscle regions (posterior-medial) furthest from the entrance of the thoracodorsal artery. Eight to 10 wk after acute muscle dissection, posterior-medial hyperemic flows were restored. Chronic stimulation of the LDM for 8–10 wk, in either dissected or nondissected muscles, did not alter regional blood flows in noncontracting muscle; however, it significantly reduced hyperemic flows in all muscle regions, although capillary density was increased and the muscle was transformed into a predominantly type I fiber type. These results, coupled with data from previous experiments, suggest that the muscle damage observed in the posterior-medial regions of the LDM after surgical dissection and chronic stimulation may be related to reduced hyperemic flow responses caused by surgical isolation of the muscle.

METHODS

All animals were treated humanely in accordance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by NIH (DHEW Publication No. (NIH) 86-23, revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892). The protocol was approved by the Animal Care Committee of the Deborah Research Institute.

Two separate studies, acute and chronic, were conducted. The acute study involved anesthetizing goats and measuring regional LDM blood flow at rest and during muscle contraction. The chronic study involved two surgical procedures. The first procedure was conducted to ligate the collateral vessels of the LDM and attach a nerve-cuff electrode for subsequent chronic stimulation of the muscle. The second procedure, which occurred 8–10 wk after the first, involved anesthetiz-
ing the goat and measuring regional LDM blood flows at rest and during muscle contraction. Histological data from the chronic study have been previously published (17).

Acute study. Male Nubian goats (36–46 kg) were anesthetized with 2% isoflurane (NarcoMed AVE-K gas anesthesia machine, North American Dräger) after premedication with ketamine (10 mg/kg im) and diazepam (0.5 mg/kg im). A cuffed endotracheal tube was inserted, and the animal was ventilated at a tidal volume of 15 ml/kg and a rate of 10–12 breaths/min. Body temperature was monitored and maintained at 37°C by using a heated surgical table. Arterial blood gases and pH were monitored at 30-min intervals. A gastric tube was inserted into the stomach, and the contents were allowed to drain freely into a bucket during the experiment. An ear vein was cannulated for administration of fluids (0.9% saline at a rate of 1 l/h). The left ventricle was catheterized via the left carotid artery for measurement of left ventricular pressure and for administration of the microspheres. The left femoral artery was cannulated as the site for the reference pressure and for administration of the microspheres. The left femoral artery was cannulated as the site for the reference withdrawal sample during microsphere injection. This site was also used to obtain arterial blood samples for blood-gas analysis. Sodium bicarbonate was given, and respiratory rate and depth were adjusted as needed to maintain arterial pH and PCO2 within a prescribed range (pH 7.36–7.40, PCO2 and depth were adjusted as needed to maintain arterial pH analysis. Sodium bicarbonate was given, and respiratory rate was also used to obtain arterial blood samples for blood-gas withdrawal sample during microsphere injection. This site femoral artery was cannulated as the site for the reference pressure and for administration of the microspheres. The left femoral artery was cannulated as the site for the reference withdrawal sample during microsphere injection. The left ventricle was cannulated as the site for the reference withdrawal sample during microsphere injection.

There were three injections of microspheres during each experiment. Each injection occurred during different experimental interventions on the right and left LDM (Table 1). Occlusion of the TDA was accomplished by careful dissection under the lateral aspect of the muscle (near to its insertion) and exposure of a short length of the TDA, which was then occluded with a nontraumatic vascular clamp. To occlude all other vascular supplies to the muscle (posterior-medial, secondary supplies), the muscle was surgically dissected from tissue attached on the anterior and posterior aspects of the muscle as well as from attachments to underlying muscles. This latter dissection was accompanied by ligation of two to three major arterial-venous vessel pairs that entered the posterior-medial aspect of the LDM from underlying muscle. Muscle contraction was accomplished by placing a nerve-cuff electrode (model 4080, Medtronic) around the motor nerve bundle entering the muscle near the TDA and stimulating the nerve electrically by using a programmable pulse generator (Itrel-2 model 7421, Medtronic) with 30-Hz bursts (190 ms on, 500 ms off) that elicited muscle contractions at a frequency of 87 contractions/min. A supramaximal voltage was used (generally 5–7 V). Contraction was maintained for 3 min 10 s. Ninety seconds after LDM contraction was initiated, microspheres were injected, which required 100 s for withdrawal of the reference sample, followed by termination of the contraction. When contraction was accompanied by TDA occlusion, the occlusion period began 10 min before the contractions were initiated. When blood flow was measured during TDA occlusion alone, the occlusion was in place for 10 min before microspheres were injected. TDA occlusions were removed after the arterial reference sample was obtained.

Microsphere technique. Regional blood flows were determined by using colored microspheres (red, blue, and yellow Dye Trak microspheres; 3 × 10⁶ spheres/ml; Triton Technology, San Diego, CA) with an average diameter of 15 µm. Before being injected into the animal, the microspheres were vortexed for at least 60 s to ensure dispersal. Microspheres (6.67 ml containing 20 × 10⁶ spheres) were injected into the left ventricular catheter over 10 s, followed by a saline flush (5–10 ml) lasting 10 s. The syringe withdrawal pump was turned on 10 s before microsphere injection at a rate of 10 ml/min. The total withdrawal time was 100 s. The volume withdrawn was noted, and the blood sample was aliquoted into four plastic vials and placed on ice until processed.

At the conclusion of the experiment, muscle samples were obtained from the left and right LDM in all five goats. Total LDM weights ranged between 113 and 187 g. Ten samples were obtained from each muscle at the locations designated in Fig. 1. Each sample was then divided into two portions and weighed (range 0.6–2.5 g). Blood flows were determined from each sample pair by using the colored-microsphere technique, and the results were averaged for that sample number. In the statistical evaluation of the blood flows, samples were combined to reflect five muscle regions with region 1/2 nearest the insertion and region 9/10 nearest the origin along the spine (Fig. 1).

Anatomically, region 1/2 was closest to the TDA (lateral muscle) and regions 7/8 and 9/10 (medial muscle) were closest to the entrance of secondary, distal vascular supply to the muscle, which was typically composed of two to three separate arterial-venous pairs entering the muscle in region 8.

Chronic study. Ten castrated male Nubian goats (25–47 kg) were premedicated with ketamine (10 mg/kg im) and diazepam (0.5 mg/kg im) and anesthetized with isoflurane (1.5–

<table>
<thead>
<tr>
<th>Injection</th>
<th>Right LDM</th>
<th>Left LDM</th>
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<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>TDA occlusion</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>TDA occlusion</td>
</tr>
<tr>
<td>3</td>
<td>Posterior-medial ligation</td>
<td>Posterior-medial ligation</td>
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LDM, latissimus dorsi muscle; TDA, thoracodorsal artery.

Fig. 1. Latissimus dorsi muscle (LDM) regions sampled for determination of regional blood flows by colored-microsphere method. Thoracodorsal artery enters muscle in region 1/2 and distal vascular supply enters at region 8, typically.
2.0%; NarcoMed AVE-K, North American Dräger). The animals were intubated and mechanically ventilated at 400-ml tidal volume and 18 breaths/min. Body temperature and arterial blood gases were maintained as in the acute study. Sterile procedure was used throughout the operation. An incision was made that extended along the lateral and posterior borders of the LDM from the axillary fold to the costal margin of the 11th rib. In one group of goats (n = 5), the thoracodorsal neurovascular bundle was carefully dissected on the right side, a nerve-cuff electrode (model 4080, Medtronic) located in a subcutaneous pocket. This muscle group was designated as nondissected and stimulated LDM (group S). The left LDM was surgically dissected from surrounding tissues with ligation of all collateral vessels, which consisted of one to three vessels located in the middle and distal aspect of the muscle, and the nerve-cuff electrode was placed around the TDN but was not connected to a pulse generator (group D, dissected only). In the second group of goats (n = 5), the left LDM was completely dissected from surrounding tissues with ligation of all collateral vessels and the anterior border of the LDM dissected free from the underlying musculature while the origin and insertion of the LDM were left intact. A nerve-cuff electrode was placed around the TDN and connected to a pulse generator (group DS, dissected and stimulated). The right LDM had a nerve cuff placed around the TDN but was not dissected or stimulated (group N). In all the dissected muscles, the TDA and TDN (i.e., the thoracodorsal neurovascular bundle), the aponeurosis origin, and the muscle insertion were left intact. During closure, the lateral and posterior borders of the LDM were carefully sutured to the underlying musculature by using nonabsorbable sutures (O-coated VICRYL) so as to attach the muscle precisely in its original anatomic location. After a 14-day recovery period, the pulse-generator program was activated with the following parameters: amplitude, 5 V; pulse width, 210 µs; burst frequency, 10 Hz for the first 2 wk of stimulation and 30 Hz thereafter; burst duration, on time of 190 ms and off time of 500 ms. After the chronic muscle stimulation period of 8–10 wk, the animals were anesthetized as before and muscle blood flows at rest and during stimulation were determined by using colored microspheres.

The second procedure in the chronic study was very similar to the acute study. Briefly, the goat was anesthetized with isoflurane after premedication with ketamine, intubated, and placed in a ventilator, and a gastric tube was inserted. A catheter was positioned in the left ventricle via the carotid artery for microsphere injection, and a femoral artery was cannulated for the reference blood withdrawal and for obtaining blood samples for blood-gas measurements.

There were two injections of microspheres during each experiment. The first injection (yellow microspheres) was conducted 30 min after the Itrel pulse generators were deactivated so that resting LDM blood flow could be obtained. The second injection (blue microspheres) occurred 5 min after initiation of muscle contraction by using the same stimulation parameters used during chronic stimulation. The procedures followed for the microsphere injections were identical to those used in the acute study.

At the conclusion of the experiment, muscle samples were obtained from both LDMs in all 10 goats. The total muscle weights ranged between 69 and 164 g. Muscle weights relative to body weights were previously reported (17). Muscle samples (1.1–3.7 g) were obtained from three muscle regions for blood flow determinations (proximal, middle, and distal, relative to the muscle insertion onto the humerus). The proximal region corresponds to regions 1–4 (Fig. 1), the middle region to regions 5–8 (Fig. 1), and the distal regions to region 9/10 (Fig. 1).

Microsphere processing. The reference blood samples and muscle biopsy samples were processed as follows for determination of blood flow. KOH (4 M) containing 0.05% Tween 80 was added to the tissue samples (previously trimmed of fat and weighed), and 16 M KOH were added to the blood (equal volumes of KOH to the blood and 7 ml of KOH to the tissue samples). The samples were digested at room temperature until digestion was complete. The digested tissue solution was heated to 72°C for 2 h and quickly vacuum filtered (10-µm pore size; 25-mm-diameter filter). Ethanol (70%) was used to wash the filtered spheres. The washed and dried spheres (with the filter paper) were then placed into a 1.5 ml Eppendorf tube to which 300 µl of N,N-dimethylformamide (DMF) were added to extract the dye. After vortexing and centrifugation (2,000 g, 100 µl of the DMF-dye solution were transferred to a spectrophotometer cuvette. The extracted dye was scanned on a spectrophotometer (model DU640, Beckman) at wavelengths between 350 and 820 nm. The absorption spectrum was saved as an ASCII file and transferred to a computer running the MISS program (Triton Technology). This program separates the individual components of the compound absorbance spectra, which has overlapping spectra of the different dyes. The reference samples, tissue samples, and standards spectra, along with tissue weights, were entered into the program for computation of blood flow. The sample blood flow values were entered into a spreadsheet and expressed as milliliters per minute per 100 g tissue weight. When only two microsphere colors were used, which did not have overlapping spectra (blue and yellow microspheres), the peak absorbance at the specified wavelength was entered into an Excel spreadsheet for computation of flow instead of using the MISS program.

Capillary density determination. Capillaries were stained for alkaline phosphatase by using a modified procedure as described by Yang et al. (31).

Statistical evaluation. The data were analyzed by one-way analysis of variance followed by the Dunnett’s multiple-comparison test using InStat (Graphpad Software, San Diego, CA).

RESULTS

Acute study. The control resting blood flow, averaged for all five muscle regions in 5 LDMs was 2.3 ± 0.2 ml·min⁻¹·100 g⁻¹ (Fig. 2). Occlusion of the TDA did not significantly alter whole muscle blood flow (2.7 ± 0.2 ml·min⁻¹·100 g⁻¹). Ligation of the posterior-medial
blood supplies caused whole muscle blood flow to increase to 7.7 ± 0.5 ml·min⁻¹·100 g⁻¹ (P < 0.05 relative to control resting flow group). This increase in blood flow may have been due to acute surgical trauma or to sympathetic denervation. Therefore, removal of either the lateral (TDA) or posterior-medial vascular supplies did not reduce whole muscle resting blood flow. Furthermore, resting blood flows within the five muscle regions were not significantly different in resting control muscles, TDA-occluded muscles, or posterior-medial-occluded muscles (Fig. 3). This indicated that adequate collaterals existed to maintain normal resting flows despite complete occlusion of either the TDA or posterior-medial vascular supplies. Muscle having the posterior-medial vascular supply ligated, however, had significantly higher resting flows (2- to 4-fold) in all regions compared with the control and TDA-occluded muscles (Fig. 3). The higher resting flows in this group resulted from all the regions in one muscle having particularly high resting flows (~15 ml·min⁻¹·100 g⁻¹).

Muscle contraction induced by electrical stimulation of the nerve caused a large hyperemic response measured 3 min after contraction was initiated. In control muscles having an intact vascular supply, muscle contraction for 3 min caused whole muscle blood flow to increase to 64.5 ± 0.9 ml·min⁻¹·100 g⁻¹ (Fig. 2). Occlusion of the TDA significantly reduced the whole muscle hyperemia to 26.3 ± 8.7 ml·min⁻¹·100 g⁻¹. Therefore, the posterior-medial vasculature supplied 41% of the total muscle blood flow during contraction. Ligation of the posterior-medial vascular supply limited the increase in blood flow during contraction to 43.0 ± 10.3 ml·min⁻¹·100 g⁻¹. Therefore, the TDA supplied 67% of the total muscle blood flow during contraction under these experimental conditions. Removal of either the TDA or posterior-medial vascular supply to the LDM significantly diminished by 30–60% the increase in blood flow associated with muscle contraction. This indicates that both vascular supplies (TDA and posterior-medial) are required to achieve normal hyperemic responses to contraction, as would need to occur with cardiomyoplasty.

The effects of vascular occlusion on active hyperemia were even more pronounced when the five regional blood flows were compared. Occlusion of the TDA during muscle contraction severely compromised active hyperemia in regions 1/2 (8.2 ± 3.1 ml·min⁻¹·100 g⁻¹), 3/4 (8.3 ± 3.4 ml·min⁻¹·100 g⁻¹), and 5/6 (21.0 ± 6.9 ml·min⁻¹·100 g⁻¹), which are in the lateral and middle regions of the muscle near the entrance of the TDA into the muscle (Fig. 4). These muscle regions when left intact had hyperemic flows >60 ml·min⁻¹·100 g⁻¹. These reduced hyperemic flows, however, were still above control resting flow, which averaged ~2 ml·min⁻¹·100 g⁻¹ in these regions (Fig. 3). In contrast, active hyperemia in the posterior-medial regions (7/8 and 9/10) was maintained during TDA occlusion.

Removal of the posterior-medial vascular supply resulted in greatly diminished active hyperemia in the distal regions of the muscle. The hyperemic flows were 15.5 ± 2.4 and 23.3 ± 5.1 ml·min⁻¹·100 g⁻¹ in regions 7/8 and 9/10, respectively. These reduced hyperemic flows, nevertheless, were above control resting flows in these same regions (Fig. 3). The flow responses in the lateral regions (1/2 and 3/4) and middle muscle regions (5/6) were essentially normal (50–70 ml·min⁻¹·100 g⁻¹). Therefore, removal of the posterior-medial vascular supply to the LDM during surgical dissection, while not affecting resting flow, prevents normal hyperemic responses of the medial muscle regions during muscle contraction.

Chronic study. Resting blood flow in control muscles (group N) was 6.5 ± 0.7 ml·min⁻¹·100 g⁻¹ (Fig. 5) and was the same in the proximal, middle, and distal muscle regions (Table 2). LDM resting blood flows 8–10 wk after surgical dissection (group D), dissection plus chronic stimulation (group DS), or chronic stimulation without dissection (group S) were not different among the groups (Fig. 5). Furthermore, there were no regional differences in resting flows among the groups (Table 2). Therefore, none of the interventions (dissection, chronic stimulation, or both combined) altered resting blood flows.

Blood flow during muscle contraction in control muscles (group N) increased 15-fold to ~100 ml·min⁻¹·100 g⁻¹ (Fig. 5). The magnitude of active
hyperemia was similar in the three muscle regions (Table 2). Surgical dissection alone (group D) did not significantly reduce total muscle blood flow or regional flows during contraction, although there was a tendency for the active hyperemia to be reduced in the middle and distal muscle regions. When the surgical dissection was combined with chronic stimulation (group DS), there was an overall reduction in muscle blood flow during contraction, with the reduction being similar in all three muscle regions. In these muscles, contraction resulted in only a threefold increase in flow. Chronic stimulation alone, without surgical dissection (group S), also resulted in reduced hyperemic flows.

In control muscles, the capillary density was 308 ± 28/mm² and the capillary-to-fiber ratio was 1.01 ± 0.04 capillaries/fiber (Fig. 6). Muscle dissection alone (group D) did not alter capillary numbers; however, chronic stimulation with or without dissection (groups DS and S, respectively) resulted in a significant increase in the capillary number expressed either as capillary-to-fiber ratio or capillaries per square millimeter, both of which increased ~60% (Fig. 6). There were no differences in capillary numbers among the proximal, middle, and distal regions of the muscle in any treatment group. Therefore, chronic stimulation for 8–10 wk increased the total number of capillaries throughout the LDM and the number of capillaries around each muscle fiber. Furthermore, this increase in capillary density was not altered by surgical dissection and removal of posterior-medial vascular supplies to the LDM.

DISCUSSION

A major concern in using the LDM in cardiomyoplasty is that the surgical dissection of the muscle results in the ligation of several arterial sources for perfusing the LDM, particularly the posterior-medial regions of the muscle (26–28, 30). If ischemic regions do not have blood flow restored through angiogenesis or collateralization, then necrotic changes will diminish the ability of those muscle regions to contract when wrapped around the heart. We determined that acute surgical dissection of the muscle, which included disruption of all vascular supply to the muscle except for that derived from the TDA, did not reduce blood flow in any muscle regions when the muscle was at rest. Therefore, adequate interconnections (collaterals) exist between the TDA and posterior-medial arterial supplies to ensure normal perfusion (as determined by the microsphere technique) when the secondary vascular supplies are removed. It is important to emphasize, however, that “resting” flow as defined in this study is the flow value found in noncontracting muscle of anesthetized goats and, therefore, may be significantly lower than “resting” flow found in the conscious animal (2, 20).

Although resting blood flows were not reduced by acute surgical dissection, the hyperemic response during muscle contraction was greatly diminished, particularly in the medial portions of the muscle that are used to wrap the heart in the cardiomyoplasty procedure. This could be of critical significance to the preservation of the dissected muscle because resting flow requirements are higher in the conscious animal (20, 21). Furthermore, normal postural and locomotory activities of the goat will elicit hyperemic responses that may not be adequately met in the dissected muscle until collateralization occurs. When blood flows were evaluated 8–10 wk after surgical dissection, resting blood flows were still normal in the three muscle regions, and

Table 2. Blood flows in proximal, middle, and distal regions of LDM after 8–10 wk of chronic stimulation

<table>
<thead>
<tr>
<th>Group</th>
<th>Proximal</th>
<th>Middle</th>
<th>Distal</th>
<th>Proximal</th>
<th>Middle</th>
<th>Distal</th>
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<tbody>
<tr>
<td>N</td>
<td>6.7 ± 0.7</td>
<td>6.6 ± 1.2</td>
<td>6.3 ± 0.9</td>
<td>105.6 ± 21.5</td>
<td>98.4 ± 23.7</td>
<td>94.1 ± 12.6</td>
</tr>
<tr>
<td>D</td>
<td>6.1 ± 1.2</td>
<td>4.6 ± 1.2</td>
<td>8.7 ± 1.7</td>
<td>92.1 ± 25.0</td>
<td>54.4 ± 0.6</td>
<td>53.9 ± 15.9</td>
</tr>
<tr>
<td>DS</td>
<td>7.6 ± 1.5</td>
<td>12.4 ± 5.1</td>
<td>15.2 ± 6.6</td>
<td>29.7 ± 3.9*</td>
<td>34.2 ± 9.4*</td>
<td>27.5 ± 8.0*</td>
</tr>
<tr>
<td>S</td>
<td>7.3 ± 1.7</td>
<td>6.9 ± 1.7</td>
<td>5.2 ± 1.6</td>
<td>37.3 ± 7.8*</td>
<td>55.5 ± 8.2</td>
<td>34.2 ± 12.8*</td>
</tr>
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</table>

Values are means ± SE for 5 muscles in each group except for group D, where there were 4 muscles. Group N, nondissected and nonstimulated; group D, dissected and nonstimulated; group DS, dissected and stimulated; group S, nondissected and stimulated. Within each group, there was no significant difference in regional blood flows. Within each region, there were no significant differences between groups for resting flows; however, there were significant differences for contracting flow relative to group N (*P < 0.05).
unlike the findings in the acute study, the active hyperemic response to muscular contraction was not significantly impaired. This suggests that angiogenesis or collateralization occurs over the 8- to 10-wk period, which restores much of the acute loss of the hyperemic response resulting from muscle contraction.

Cardiomyoplasty has been used successfully in human patients as well as in animal experiments (7, 8, 10, 22). To use skeletal muscle to provide mechanical assistance to the failing heart, it is necessary to use a muscle that is resistant to fatigue during chronic repetitive stimulation. The muscle needs to be slow twitch (type I), highly oxidative, and accessible for wrapping around the heart. There are no muscles near to the heart (except for the diaphragm and pectoralis major) that can be used for this purpose unless they are first transformed from a mixture of type I and II fiber types to type I. It has been shown that the LDM is well suited for this use and with chronic stimulation can be transformed into a highly fatigue-resistant type I muscle (8, 9, 12, 16, 17, 22, 25). It has been documented that the LDM exhibits muscle damage and necrosis in the very regions that are used to wrap the heart (12, 17, 22). This damage can result from surgical trauma, ligation of arterial supplies to the muscle, mechanical obstruction of the blood flow as the muscle enters the thorax, chronic stimulation and contraction of the LDM, change in muscle length and/or tension, or a combination of any or all of these factors. The ischemic-injury hypothesis is quite tenable because the surgical isolation and translocation of the muscle require ligating several arterial vessels that supply the muscle, particularly the posterior-medial regions. Mannion et al. (26) and Isoda et al. (19) found by using the dog LDM that ligation of these vessels reduced resting flow and active hyperemia, particularly within the medial regions of the muscle. The technique used by Isoda et al. for measuring regional blood flows (He-Ne laser blood flowmeter), however, gave questionable results because, although the resting blood flow values were normal, the increases in regional blood flows during contraction were only 1–2 ml·min⁻¹·100 g⁻¹. Total muscle flows measured at the TDA increased by >15 ml·min⁻¹·100 g⁻¹ during contraction. This lack of agreement between regional flows and total flows when expressed per the same unit tissue weight suggests that the regional flow measurement technique was not accurately measuring blood flow. Mannion et al. (26) used radiolabeled microspheres and clearly showed reductions in both resting and hyperemic flows in the medial regions of the dog LDM. Our study has shown that ligation of these vessels does not reduce resting blood flow to any region of the muscle, either acutely or 8–10 wk after ligation. Therefore, the TDA is able to supply all of the resting blood flow needs of the muscle, at least in the anesthetized goat.

We found that the resting blood flow averaged ∼3–6 ml·min⁻¹·100 g⁻¹ throughout the muscle. These resting flow values are in the range of those observed in resting skeletal muscle of other species when anesthetized (5, 11, 14, 15, 19, 21, 23). Because the oxygen requirements of resting skeletal muscle are low (5, 11, 14, 15), and oxygen extraction is low, these flows are sufficient to prevent tissue hypoxia in noncontracting muscle. One caveat to this is that our microsphere technique for assessing blood flow used tissue samples of 1–3 g in weight, and flow heterogeneity within the sample cannot be assessed. While the average blood flow within these muscle samples was 3–6 ml·min⁻¹·100 g⁻¹, there may have been regions within the sample that had subnormal flows. Some muscle fibers may have been ischemic, which would lead to damage (17), while other fibers may have been supplied with elevated blood flow under resting conditions. Experiments need to be conducted to address the issue of flow heterogeneity after surgical dissection. This could be done by quantifying the number of functional (perfused) capillaries.

Although the acutely dissected LDM was not ischemic at rest (as determined within the limits of our technique), we found that blood flow during contraction was significantly compromised in the posterior-medial regions of the muscle. Ordinarily, when skeletal muscle contracts, vascular resistance falls and blood flow increases (active hyperemia) (5, 11, 14). A tight coupling normally exists between tissue oxygen consumption and blood flow in contracting muscle to maintain adequate oxygen supply to the tissue during contraction (4, 11). The acutely dissected LDM was unable to increase blood flow during contraction to the same degree as was nondissected muscle because its posterior-medial arterial supply was removed. Resting flow in the posterior-medial regions was normal probably because autoregulatory responses, and perhaps opening of preexisting collaterals, reduced vascular resistance within those regions to maintain flow. This fall in resistance, however, would diminish the vasodilatory capacity of these regions of the LDM during contraction, resulting in a reduction in the magnitude of active hyperemia. If the muscle oxygen consumption remained the same during contraction in dissected and nondissected muscles, then the reduced active hyperemia in the posterior-medial regions would lead to an insufficient oxygen supply and tissue hypoxia during contraction. Reduced hyperemic capacity in the dissected muscle could also result in inadequate blood flow during normal postural and locomotory activities where the blood flows are considerably higher than those observed in muscles from anesthetized animals (2, 20). Without adequate oxygen delivery, muscle contractile performance will be impaired and degenerative changes might occur. However, 3 mo after surgical dissection, the active hyperemic response was not significantly diminished, indicating that the hyperemic response, which was severely reduced after acute dissection, was restored. This restoration of hyperemic response (in nontransformed muscles) may have been due to opening of collaterals or formation of new collaterals by angiogenesis. Mannion et al. (26) observed a similar restoration of hyperemic flow 3 wk after vessel ligation in the dog LDM.
The medial regions of the goat LDM have been found to undergo degenerative changes 8–10 wk after surgical dissection (17). The present study suggests that this is probably not due to altered resting blood flow, unless there was flow heterogeneity within the samples. Because the degenerative changes were found predominantly in the distal (medial) regions of the LDM, this suggests that the degeneration was somehow associated with the removal of the secondary, distal vascular supply to the muscle. Although resting flow was not compromised either acutely or chronically, the hyperemic response to contraction was compromised, at least initially after the surgical dissection. Perhaps this initial reduction in flow capacity rendered the distal LDM regions hypoxic when the muscle contracted during normal postural and locomotory activities during the 8- to 10-wk postsurgical period, and this precipitated the degenerative changes in the muscle. Distal degeneration of the muscle (17) would also explain the tendency for the distal hyperemic response to be lower 8–10 wk postsurgery.

Chronic stimulation and transformation of the LDM in the absence or presence of surgical dissection resulted in decreased hyperemic blood flows at a given frequency of muscle contraction. Chronic stimulation alone does not cause significant muscle damage (17); therefore, damage cannot be the cause of the reduced functional hyperemia after chronic stimulation. The most likely explanation is that chronic stimulation transforms the LDM from a type II to a type I muscle (8, 9, 12, 16, 17, 22, 25). By the end of the 8- to 10-wk period of chronic stimulation, the type I fiber percent increased from ∼35 to 95% (varied among muscle regions) (17). Type I fibers generate less force during contraction and use ATP more efficiently than do type II fibers (3) and therefore would consume less oxygen, thereby requiring a smaller increase in blood flow to supply oxygen to the tissue. In a study by Acker et al. (1), dog LDM transformed by electrical stimulation in a manner similar to that in our study had significantly reduced hyperemic flows for a given tension-time index, suggesting increased efficiency. Mannion et al. (26) also noted decreased hyperemic responses in electrically transformed dog LDM when contracting at different frequencies. On the other hand, it should be noted that muscles transformed by exercise training rather than electrical stimulation generally show increased hyperemic responses at a given frequency of tetanic trains of stimulation in situ (24).

It is well established that highly oxidative muscle fibers have more capillaries than do low-oxidative fibers (13, 29) and that chronic electrical stimulation induces angiogenesis (6). The chronic burst-stimulation protocol used in this study induced a 64% increase in capillary-to-fiber ratio in the nondissected and stimulated LDM, which compares favorably with the 75% increase in capillary-to-fiber ratio in rabbit fast-twitch muscle that was stimulated for 28 days by using a continuous 10-Hz frequency (6). Capillary-to-fiber ratios have been shown to have a high correlation with the oxidative capacity of muscle (13). In this study, the nearly twofold increase in capillary-to-fiber ratio was associated with an approximate twofold increase in oxidative capacity (17). The capillary density increased from 308 ± 28 capillaries/mm² in control muscles to 542 ± 28 capillaries/mm² in chronically stimulated muscles (76% increase). This percent increase is slightly greater than the percent increase in capillary-to-fiber ratio because of the smaller fiber diameter in the transformed muscle (17).

In summary, we have shown that surgical dissection of the LDM in anesthetized goats reduces acutely the hyperemic response to muscle contraction in the posterior-medial regions of the muscle without affecting resting blood flow. This reduction in hyperemic flow may have deleterious consequences (e.g., enhance muscle damage) when an electrical stimulation protocol is imposed on the muscle that results in a large increase in oxygen demand that cannot be met by the blood supply. Furthermore, hyperemic responses to normal postural and locomotory activity might also be impaired in the dissected muscle, which will further enhance muscle damage. The hyperemic response is restored to a large extent 8–10 wk later; however, the posterior-medial regions of the muscle are already damaged at this time (17). Transformation of the LDM from a type II to a type I muscle by using chronic stimulation, in surgically dissected or intact muscles, resulted in a greatly diminished active hyperemic response throughout the LDM and is probably a consequence of the change in fiber type to a more efficient oxidative type I fiber composition within the LDM.

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